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A Parallel Reaction Monitoring (PRM) Case Study: Targeted Quantitation of AKT Isoforms

Skeletal muscle physiology and metabolism are regulated by complex networks of intracellular signaling pathways.

Among many of these pathways, the protein kinase AKT plays a prominent role.

While three AKT isoforms have been identified (AKT1, AKT2, and AKT3), surprisingly little is known regarding isoform-specific expression of AKT in human skeletal muscle.

To address this, we examined the expressions of each AKT isoform in muscle biopsy samples collected from the vastus lateralis of healthy male adults at rest.

Methods

Bioinformatics and peptide mapping was used to identify peptides unique to each AKT isoform. The peptides are highlighted below.

```
AKT1 ELILMEEIRFPRTLGPEAKSLLSGLLKKDPKQRLGGGSEDAKEIMQHRFFAGIVWQHVYE
AKT2 ELILMEEIRFPRTLSPEAKSLLAGLLKKDPKQRLGGGPSDAKEVMEHRFFLSINWQDVVQ
AKT3 ELILMEDIKFPRTLSSDAKSLLSGLLIKDPNKRLGGGPDDAKEIMRHSFFSGVNWQDVYD
       ******:*:*****. :*****:*** ***::***** .****:*.* ** .: **.* :
```

A sensitive and selective Parallel Reaction Monitoring (PRM) assay was developed. The method details are included in the table below.

Target Protein	Peptide Sequence	Precursor ion *m/z*	Product ion *m/z*		
AKT1	SLLSGLLK	415.7709	173.128	517.418	630.418
	SLLSGLLK[HeavyK]	419.778	173.128	525.348	638.432
AKT2	SLLAGLLK	407.7735	173.128	501.339	614.423
	SLLAGLLK[HeavyK]	411.7806	173.128	509.353	622.437
AKT3	SLLSGLLIK	472.313	173.128	630.418	743.502
	SLLSGLLIK[HeavyK]	476.3201	173.128	638.432	751.516

The method was applied to the human tissue biopsies. A PAN-AKT antibody was used a first step enrichment of the target proteins. Extracts were digested with trypsin, spiked with stable labeled internal standard peptides and analyzed using the targeted method.

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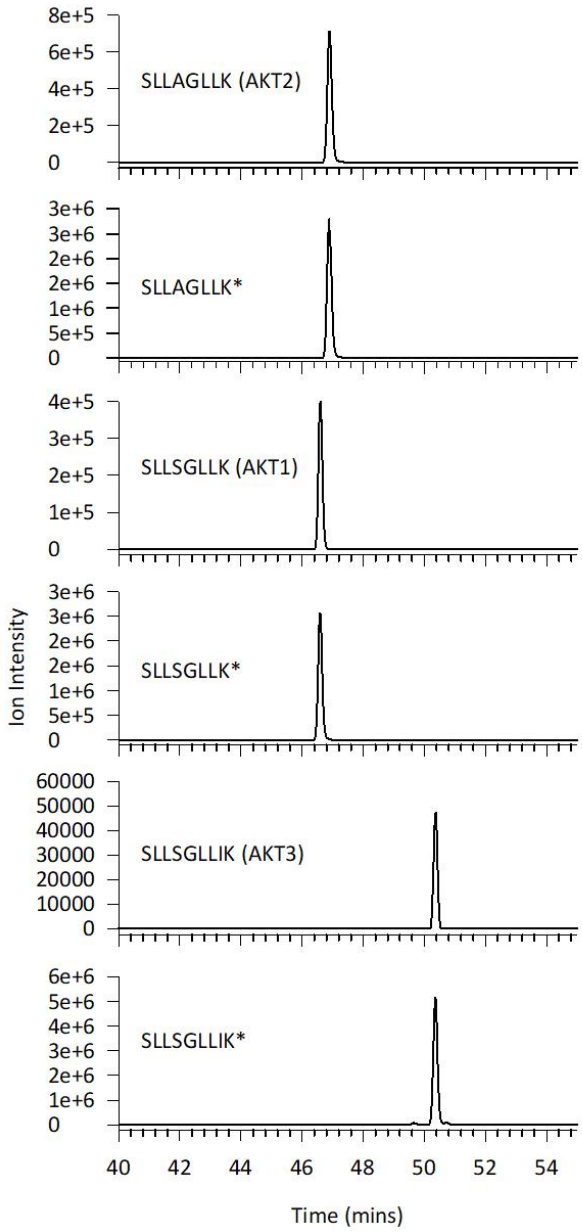
Results

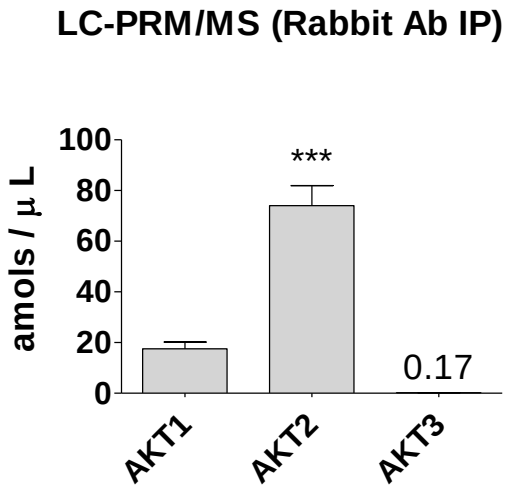


Representative chromatograms for AKT isoforms and the stable labeled internal standard peptide (*) are shown opposite.

The LC-PRM/MS method was applied to eluates derived from immunoprecipitations of human vastus lateralis lysates using either rabbit or mouse pan AKT antibodies, as indicated (means ± SEMs; n=4 subjects; *, P<0.05 vs. AKT1 and AKT3; ***, P<0.001 vs. AKT1 and AKT3).

Conclusion

AKT2 as the most highly-expressed AKT isoform in human skeletal muscle



Summary

More details are available in the Open Access article: “AKT2 is the predominant AKT isoform expressed in human skeletal muscle”. Physiological Reports, R.W. Matheny et al. 2018

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